

Simultaneous determination of losartan and hydrochlorothiazide in tablets by high-performance liquid chromatography

Giuseppe Carlucci ^{a,*}, Giancarlo Palumbo ^b, Pietro Mazzeo ^a,
Maria Giovanna Quaglia ^c

^a *Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università dell'Aquila, Via Vetoio, 67010 Coppito (L'Aquila), Italy*

^b *Dipartimento di Medicina Interna e Sanità Pubblica, Università dell'Aquila, Via S. Sisto, 67100 L'Aquila, Italy*

^c *Dipartimento di Studi Farmaceutici, Università 'La Sapienza', P.le Aldo Moro 5, 00185 Rome, Italy*

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Abstract

A method for the simultaneous determination of losartan potassium and hydrochlorothiazide in tablets is described. The procedure, based on the use of reversed-phase high-performance liquid chromatography, is linear in the concentration range 3.0–7.0 $\mu\text{g ml}^{-1}$ for losartan and 0.5–2.0 $\mu\text{g ml}^{-1}$ for hydrochlorothiazide, is simple and rapid and allows accurate and precise results. The limit of detection was 0.08 $\mu\text{g ml}^{-1}$ for losartan and 0.05 $\mu\text{g ml}^{-1}$ for hydrochlorothiazide. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Losartan, the potassium salt of 2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole (Fig. 1), is the prototype of a new generation of effective and orally active nonpeptide angiotensin II receptor antagonists. These substances have been developed in sequence to the angiotensin converting enzyme inhibitors as a further therapeutic action

on the renin-angiotensin-aldosterone system, one of the most important regulators of blood pressure [1,2]. 5-carboxylic acid metabolite of losartan itself is a potent angiotensin II antagonist [3–5].

Hydrochlorothiazide, or 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Fig. 1), is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance.

The two drugs are successfully used in association in the treatment of hypertension [6–11].

* Corresponding author. Tel.: +39-862-4311; fax: +39-862-433753.

E-mail address: carlucci@aquila.infn.it (G. Carlucci)

The determination of losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography [12,13], in urine by gas chromatography-mass spectrometry [14], and, simultaneously with its active metabolite in biological fluids, by HPLC [15–20].

Several analytical procedures have been described for the individual determination of hydrochlorothiazide, most frequently by using electrochemical or spectrophotometric methods [21], and jointly with other pharmaceutical substances, including spectrophotometric [22–28] and HPLC [24,29–31] procedures.

So far no method for the simultaneous determination of these drugs in pharmaceutical forms or in biological fluids has been described. In chemical literature is only available a procedure for the determination of hydrochlorothiazide in human plasma and urine in the presence of losartan, without any information on the quantitation of this last drug [32].

This paper describes a method for the simultaneous determination of hydrochlorothiazide and losartan potassium in tablets. The procedure, based on the use of reversed-phase high-performance liquid chromatography, is simple and rapid and provides accurate and precise results.

2. Experimental

2.1. Materials

Losartan potassium and furprofen were kindly supplied by the Department of Medicina Interna of the University of L'Aquila; hydrochlorothiazide

was purchased from Sigma-Aldrich (Milan, Italy). Acetonitrile (HPLC grade) and all other analytical-grade reagents were obtained from Farmitalia-Carlo Erba (Milan, Italy). Water (HPLC grade) was obtained by distillation in glass and passage through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Chromatographic system and conditions

HPLC analysis was carried out using a Waters (Waters, Milford, MA, USA) system composed of the following: a Model 510 pump, a Model 484 LC variable wavelength absorbance detector connected to a Model 740 Data Module integrator. A Model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 20 μ l loop was used.

The analysis was performed on an analytical 125 \times 4.0 mm I.D. reversed-phase Erbasil (5 μ m particle size) column (Farmitalia-Carlo Erba, Milan, Italy), protected by a 20 \times 4.6 mm I.D. disposable (40 μ m particle size) Pelliguard precolumn (Supelco, Bellefonte, PA, USA). Separations were performed at room temperature.

The mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH 4.0; 0.1 M) (35:65, v/v). Phosphate buffer prior to use was filtered through an HA 0.45 μ m filter, while acetonitrile through a FA 0.5 μ m filter (Millipore, Bedford, MA, USA). The mobile phase was prepared daily, sonicated before use and delivered at a flow rate of 1.0 ml min⁻¹. Column eluate was monitored at 230 nm.

2.3. Standard solutions

A stock solution containing losartan potassium (2.5 mg ml⁻¹) was prepared by dissolving a weighed amount of substance in acetonitrile. The stock solution of hydrochlorothiazide (1.25 mg ml⁻¹) was obtained by dissolving the weighed drug in the mobile phase. Standard solutions were prepared by dilution of the above stock solutions with mobile phase and by varying the losartan concentration in the range 3.0–7.0 μ g ml⁻¹ (maintaining the hydrochlorothiazide concentration at a constant level of 1.25 μ g ml⁻¹) and the hydrochlorothiazide concentration in the range

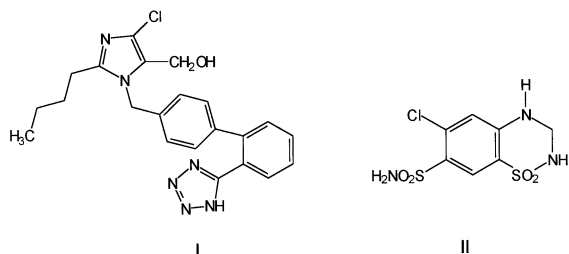


Fig. 1. Chemical structure of losartan (I) and hydrochlorothiazide (II).

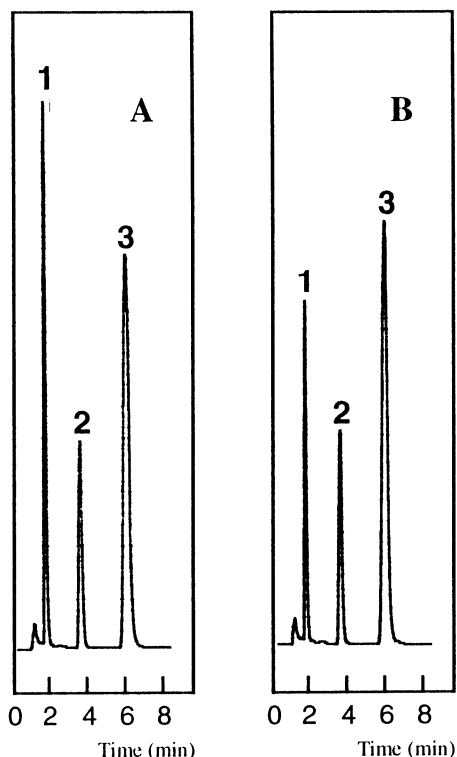


Fig. 2. HPLC profiles of a standard solution (A) and a commercial sample (B). (1) Hydrochlorothiazide; (2) furprofen (internal standard); (3) losartan. Vertical axis: UV detector response at 230 nm in the arbitrary units of 740 Data Module integrator. Hydrochlorothiazide concentration in A: $2.0 \mu\text{g ml}^{-1}$; losartan concentration in A: $5.0 \mu\text{g ml}^{-1}$.

$0.5\text{--}2.0 \mu\text{g ml}^{-1}$ (maintaining the losartan concentration at a constant level of $5.0 \mu\text{g ml}^{-1}$).

The stock solution of internal standard (furprofen) (1.0 mg ml^{-1}) was prepared with mobile phase. An aliquot of the internal standard solution, after appropriate dilution, was added to each standard solution so as to give a final concentration of $5 \mu\text{g ml}^{-1}$.

The standard solutions could be stored at 4°C for over one month with no evidence of decomposition.

2.4. Analysis of tablets

Five tablets were crushed and combined. An amount of material was accurately weighed, added with acetonitrile and centrifuged at 4000

rpm for 15 min. The clear supernatant was completely transferred into a 100 ml calibrated flask and completed to volume with acetonitrile.

The solution obtained was diluted with the mobile phase so as to obtain a concentration of the two drugs in the range of linearity previously determined. An aliquot of the internal standard solution was added to the sample solution prior to the dilution so as to give a final concentration of internal standard of $5 \mu\text{g ml}^{-1}$. HPLC analysis was carried out on aliquots of $20 \mu\text{l}$ by using the corresponding calibration curves.

3. Results and discussion

The calibration curves for HPLC analysis were obtained by plotting the peak-area ratio of each drug to internal standard versus its concentration. The equations, obtained through regression analysis of data for the above standard solutions (each datum average of a minimum number of five determinations) were: for losartan $y = 0.66x - 0.08$ ($r = 0.9986$) and for hydrochlorothiazide $y = 0.85x + 0.01$ ($r = 0.9987$), where y is the peak-area ratio in the arbitrary units of the 740 Data Module system used and x is the drug concentration ($\mu\text{g ml}^{-1}$).

The RSD values of the slope were 1.5% (losartan) and 2.4% (hydrochlorothiazide); those of the intercept were very high (until 50%) because of their low numerical values.

Fig. 2 illustrates two typical HPLC chromatograms obtained from a standard solution (A) and from a commercial sample (B). The retention times were 1.8, 4.3 and 7.2 min for hydrochlorothiazide, furprofen (internal standard) and losartan, respectively. No interfering substance was observed in the sample chromatogram.

Table 1 shows the results obtained in the analysis of some commercial antihypertensive formulations. No potential interference may derive from their composition. A comparison with a reference determination method has not been possible because so far no other procedure for the simultaneous quantitation of these drugs has been reported.

Table 1
Results obtained in the analysis of pharmaceutical tablets

Commercial sample ^a	I Nominal (mg)	I Found (mg)	II Nominal (mg)	II Found (mg)
1	50.0	49.8	12.5	12.3
2	50.0	49.7	12.5	12.5
3	50.0	49.9	12.5	12.4

^a I: Losartan potassium; II: hydrochlorothiazide. Analyses in triplicate (RSD: 2.2% for I and 0.9% for II). Composition of the tablets: I (50.0 mg); II (12.5 mg); excipients: cellulose, lactose, starch, magnesium stearate, hydroxypropylcellulose, methylhydroxypropylcellulose, titanium oxide.

The optimization of the analytical procedure has been carried out by varying the following: reversed-phase column used, mobile phase composition, flow rate, monitoring wavelength.

The degree of reproducibility of the results obtained through small deliberate variations in method parameters and by changing instruments and operators has been very satisfactory.

The proposed method shows a good linearity in the concentration ranges examined. The RSD for losartan is comprised between 2.1 and 3.3%, for hydrochlorothiazide between 0.8 and 5.0%. The limit of detection was $0.08 \mu\text{g ml}^{-1}$ for losartan and $0.05 \mu\text{g ml}^{-1}$ for hydrochlorothiazide.

The accuracy of the method was determined by investigating with the described procedure mixtures of accurately weighed amounts of the two drugs. The relative error is variable in the range 0.03–3.0% for losartan and 0.3–3.8% for hydrochlorothiazide.

4. Conclusions

This method described for the simultaneous determination of losartan potassium and hydrochlorothiazide in antihypertensive pharmaceutical forms is very simple and rapid, provides accurate and precise results, and, through an appropriate biological sample extraction procedure, should be of value for the concomitant quantitation of the two drugs in human plasma and for the study of their pharmacokinetics.

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